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Anti-Angiogenic and Anti-Metastatic Effect of Aqueous Fraction from Euchelus Asper Methanolic Extract

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Abstract : Angiogenesis and metastasis are two of the most important hallmarks of cancer. Hence, most of the cancer therapies nowadays are multi-targeted so as to reduce resistance and have better efficacy. As synthetic molecules arise with a burden of their toxicities and side-effects, more and more research is being focussed on exploiting the vast natural resources of drugs, in the form of plants and animals. Although, the idea of using marine organisms as a source of pharmaceuticals is not new, the pace at which marine drugs are being discovered, has definitely up surged! In the present study, we have assessed the anti-angiogenic and in vitro anti-metastatic activity of aqueous fraction from the extract of marine gastropod Euchelus asper. The soft body of Euchelus Asper was extracted with methanol and named EAME. Partition chromatography of EAME gave three fractions EAME I, II and III. Biochemical analysis revealed the presence of proteins in EAME III. Preliminary analysis had revealed the anti-angiogenic activity was exhibited by EAME III out of the three fractions. Hereafter, EAME III (concentration 25µg/ml-400µg/ml) was tested on chick chorioallantoic membrane (CAM) model for the detailed analysis of its potential anti-angiogenic effect. In vitro testing of the fraction (concentration 0.25µg/ml - 1µg/ml), involved cytotoxicity by SRB assay, cell cycle analysis by flow cytometry and anti-proliferative effect by scratch wound healing assay on A549 lung carcinoma cells. Apart from this, a portion of treated CAM as well as conditioned medium from treated A549 were subjected to gelatin zymography for assessment of matrix metalloproteinases MMP-2 and MMP-9 levels. Our results revealed that EAME III exhibited significant anti-angiogenic activity on CAM which was also supported by histological observations. During histological studies of CAM, it was found that EAME III caused reduction in angiogenesis by altering the extracellular matrix of the CAM membrane. In vitro analysis disclosed that EAME III exhibited moderate cytotoxic effect on A549 cells and its effect was not dose-dependent. The results of flow cytometry confirmed that EAME III caused cell cycle arrest in A549 cell line as almost all of the treated cells were found in G1 phase. Further, the migration and proliferation of A549 was significantly reduced by EAME III as observed from the scratch wound assay. Moreover, Gelatin zymography analysis revealed that EAME III caused suppression of MMP-2 in CAM membrane and reduced MMP-9 and MMP-2 expression in A549 cells. This verified that the anti-angiogenic and anti-metastatic effects of EAME III were correlated with the suppression of MMP-2 and -9. To conclude, EAME III shows dual anti-tumour action by reducing angiogenesis and exerting anti-metastatic effect on lung cancer cells, thus it has the potential to be used as an anti-cancer agent against lung carcinoma.

Keywords : angiogenesis, anti-cancer, marine drugs, matrix metalloproteinases
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